Functional Precision Medicine Enhances Clinical Outcomes of Relapsed/Refractory Pediatric and Adolescent Cancer Patients

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Abstract

Current genomics-driven precision oncology identifies actionable mutations in < 10% of cancer patients. Pediatric cancer is especially challenging due to limited mutations and fewer genomics-guided options. Functional precision medicine (FPM) addresses this by integrating genomic profiling with rapid, high-throughput functional ex vivo drug testing on live patient-derived cells. However, there is lack of FPM prospective data showing clinical utility in pediatric cancers. In this prospective, non-randomized, single-arm study (NCT03860376), we investigated feasibility and impact of FPM in pediatric/adolescent with refractory/relapsed solid and hematologic cancers. Of 25 patients, 19 (76%) had FPM data reviewed by the FPM tumor board within four weeks (FPMTB), meeting the primary outcome of the study. Additionally, six patients received FPM-guided treatment. Among these 6 patients, 83% (5 patients) experienced a greater than 1.3-fold improved progression-free survival compared to their previous therapy, and together demonstrated a significant increase in progression-free survival and objective response rate versus physician's choice-treated patients (8 patients). Post-hoc analysis showed that patients with the same subtype of cancer do not cluster together, reinforcing the concept of optimizing cancer treatments one patient at a time (n-of-1 approach). Additionally, our study used a novel artificial intelligence/machine learning (AI/ML) platform that leveraged drug responses and sequencing data to identify novel biomarkers of drug efficacy and gain potential mechanistic insights within specific subsets of pediatric cancer patients. The findings from our proof-of-principle study illustrate the impact of FPM for relapsed/refractory pediatric/adolescent cancer patients, highlight future integrations of FPM and AI/ML, and support ongoing patient cohort expansion (NCT05857969).

Introduction

Cancer is the leading disease-related cause of death for children and teens in the United States. While there have been drastic improvements in survival for some tumor types like acute lymphoblastic leukemia, similar progress for other high-risk cancers remains challenging. Moreover, progression or relapse for most pediatric patients still correlates with poor survival, even with current standard combination therapies. Therefore, there is an urgent need to develop more effective and less toxic treatments for pediatric and adolescent cancer patients.

The current standard practice for precision oncology involves genomics-guided therapy. The widespread availability of high-throughput molecular sequencing technologies has resulted in the initiation of multiple worldwide clinical trials aimed at discovering tumor-specific mutations. However, there are unique challenges to personalizing cancer treatment through genomics for pediatric patients. Children at the highest risk of relapse are those with fewer druggable genetic features, as pediatric tumors exhibit a lower mutation rate across their genomes and display different genomic alterations than adult cancers. Importantly, pediatric cancers are often driven by structural variants and copy number alterations that can be challenging to identify and target, indicating the need for novel therapeutic options specific to pediatric patients. Furthermore, many molecularly targeted drugs lack dosage guidelines and
efficacy data in children due to the limited number of early-phase clinical trials conducted for pediatric patients. Several studies have reported that pathogenic variants can be detected in 39–50% of cases using a single platform\(^1\) or in 50% of cases when multiple sequencing platforms, such as whole exome and RNA sequencing, are combined\(^{20-23}\). For instance, the GAIN/iCat2 study trial, a multicenter prospective observational study on pediatric patients with extracranial solid tumors, showed that 240 of the 345 recruited patients (69%) had a genomic alteration with therapeutic significance\(^{24}\). However, only 29 patients (8.4%) received a matched molecularly-targeted therapy, and of those only seven patients (2%) exhibited a clinical benefit, highlighting the fact that many tumors with predicted genetic vulnerabilities do not respond to the matched targeted therapy and emphasizing the presence of complex tumor heterogeneity, clonal evolution, and other non-genetic mechanisms of adaptation and therapeutic resistance.

Genomic profiling and paired clinical outcomes data have recently been leveraged to design new integrative biomarkers of therapeutic response and disease progression through statistical analysis and machine learning methods\(^{25,26}\). In particular, recent approaches have advanced towards pan-cancer biomarkers to expand the patient population that may benefit from specific therapies\(^{27}\). However, genomic biomarkers designed to match patients with frequently used cancer drugs, including chemotherapies, are currently lacking\(^{28}\).

Functional precision medicine (FPM) is a novel and rapid approach that combines molecular profiling with direct \(\text{ex vivo}\) exposure of patient-derived live tumor samples to clinically approved drugs to identify personalized therapeutic options for individual patients. FPM goes beyond relying solely on established genomic biomarkers and instead incorporates functional drug sensitivity testing (DST) of patient-derived tumor cells to broaden the range of available treatment options\(^{29-31}\). The feasibility and clinical efficacy of FPM in adult hematological cancers has been investigated in recent FPM trials in Finland and Austria\(^{32-34}\), with both independent studies demonstrating that integration of molecular profiling and high-throughput drug response data contributes to therapeutic guidance in adult patients with hematological malignancies and provides robust data for further translational research. However, interventional FPM trials have thus far exclusively addressed hematological cancers, largely due to technical challenges regarding DST in solid malignancies. More importantly, prospective FPM studies in pediatric cancer patients are lacking.

Here, we present results from a prospective, non-randomized, single-arm clinical trial (NCT03860376) to assess the feasibility and clinical impact of implementing an FPM approach to guiding treatment decisions in children and adolescents with relapsed/refractory solid and hematologic cancers. We performed \(\text{ex vivo}\) drug testing of a comprehensive panel of 120 FDA-approved drugs. Functional drug testing and tumor panel profiling data were generated and presented to an FPM tumor board (FPMTB) to implement the results in a clinically relevant timeframe. Furthermore, we interrogated similarity of patient drug response through post-hoc clustering and correlation analysis and used a novel explainable AI/ML (xAI/ML) platform to identify novel potential multi-omics biomarkers of drug response. Our study
demonstrates, for the first time, the feasibility and efficacy of using an FPM approach to identify effective novel options for advanced cancer patients with both solid and hematological malignancies, particularly in high-risk populations such as pediatric and adolescent patients. To the best of our knowledge, this is the first pediatric cancer study to enroll both solid and hematologic cancers regardless of tumor type, and the first FPM study in the United States to generate prospective treatment data on pediatric cancer patients.

Results

Patient Enrollment and Characteristics

Between 2019 and 2022, we conducted a prospective clinical trial (NCT03860376, Fig. 1), enrolling a total of 25 pediatric/adolescent cancer patients with recurrent or refractory solid tumors (76% of enrolled patients, n = 19) or hematological malignancies (24% of enrolled patients, n = 6). The trial aimed to include patients who lacked additional standard-of-care options, and thus enrolled patients irrespective of cancer type. Clinical follow-up data was collected until May 5th, 2023. Tumor biopsies or resections were obtained from enrolled patients for *ex vivo* drug sensitivity testing (DST) and actionable mutations via genomic panel profiling (UCSF500). Results of these assays were returned to clinicians and the FPMTB for review and decision-making (Fig. 1). Among the 25 enrolled patients, DST was successfully performed on 21 of 24 patients (88%) who provided tumor tissue samples, while UCSF500 genomic tumor profiling was performed on 20 out of 24 patients (83%). One enrolled patient did not provide a tumor sample, two patients provided insufficient samples, and DST was unsuccessful for one patient (Supplemental Materials-Testing and Demographics). The median time from biopsy/surgical resection at clinic to material arrival in the processing laboratory was less than 48 hours for all patients.

At study initiation, we established the null hypothesis that < 30% of enrolled patients would be able to receive FPM data, and defined trial success (demonstration of feasibility) as at least 16 of 25 patients receiving FPM data within 4 weeks of enrollment. However, the true delivery rate was higher, as a total of 19 of 25 enrolled patients (76%, p < 0.0001, 95% confidence interval = [0.5805 to 1.000]) completed both DST and genomic profiling and results were reported to an interdisciplinary FPM Tumor Board for review (Fig. 1). Analysis of DST data alone demonstrates the true delivery rate and actionability rate was also significantly greater than 30% (21 of 25 enrolled patients (84%), p < 0.0001, confidence interval = [0.6704 to 1.0000]), highlighting the efficiency of DST in providing additional treatment options to patients.

This study strived to reproduce, to the best of our ability, an approximation of the pediatric cancer patient population in Miami-Dade County. The median age of the patient cohort was 10 years of age, ranging from 0.81 to 21 years (Fig. 2a). Of the 25 patients enrolled, 40% were female (10 patients) and 60% were male (15 patients) (Fig. 2b), resembling the national incidence of pediatric cancers 1:1.1 female to male ratio. In terms of ethnicity, three patients (12%) identified as Black or African American, seventeen patients (68%) were Hispanic (16 White or Caucasian Hispanic [64%], one Mestizo [4%]), and five patients
(20%) were non-Hispanic White (Fig. 2c). This distribution approaches the ethnic distribution of the Miami-Dade County area from which patients were accrued\textsuperscript{35}.

Enrolled patients represented the breadth of pediatric cancer indications, as the study encompassed 12 different pediatric malignancies including three acute lymphoblastic leukemias (ALL), three acute myeloid leukemias (AML), one astrocytoma (AST), one ependymoma (EP), four Ewing’s sarcomas (EWS), one glioblastoma (GBM), one malignant rhabdoid tumor patient (MRT), one medulloblastoma (MB), one neuroblastoma (NB), four osteosarcomas (OS), four rhabdomyosarcoma (RMS) and one Wilms’ tumor (WT) (Fig. 2d). Thus, all hematologic cancers were leukemias (12% each), while solid malignancies consisted of sarcoma (48%), central nervous system (CNS) tumors (20%), and kidney cancers (8%). Genomics and DST testing was successfully performed across all cancer types, with only one Ewing’s sarcoma sample failing DST (Fig. 2e).

**Functional precision medicine workflow is feasible and actionable in a clinically relevant timeframe.**

We successfully performed functional DST on 21 patient-derived tumors using a panel of 120 FDA-approved agents that included 40 formulary drugs frequently used at Nicklaus Children’s Hospital in Miami, 47 nonformulary drugs approved by the FDA for cancer treatment, and therapies under investigation in phase II and phase III pediatric cancer clinical trials (Supplemental Materials-Drug List). Functional DST analysis in all 21 tested patients (100%) showed at least one effective agent (defined as agents with drug sensitivity scores (DSS) > 10) (Supplemental Fig. 1, Supplemental Materials-DST Testing Results). In contrast, only one of 20 patients (5%) had a genomically-matched therapy approved for their specific cancer type, and four of 20 patients (20%) had genomically matched therapies with potential clinical benefit approved in other cancer types (Fig. 2f). These results demonstrate that the capability of DST to provide additional treatment options to pediatric cancer patients is far greater than the effectiveness of genomic profiling alone.

Return of DST results significantly outpaced return of genomic profiling data. Following receipt, the median time for reporting DST results to the FPMTB was nine days for hematological cancers (range: 5–17 days) and 10 days for solid tumors (range: 4–23 days) (Fig. 2g). In contrast, the median turnaround time for UCSF500 genomic profiling was 26.5 days (range: 14–63 days) (Fig. 2g). This rapid turnaround time enabled the FPMTB to promptly discuss each case using functional DST data to provide treatment recommendations to a greater fraction of patients than with genomic panel profiling alone. For pediatric/adolescent patients with aggressive disease, the speed with which recommendations were made was critical for enabling guided therapeutic decision-making.

**Patients guided by FPM have improved clinical outcomes.**

Each patient received at least two lines of treatment before enrollment in our study. Decisions by the interdisciplinary FPMTB were made for each patient independently before treatment was given. Due to the rapid disease progression observed in four patients, and the necessity of surgical intervention in two patients, a total of 14 patients received medical treatment. Of the 14 patients assessed by the FPMTB,
eight patients (57%) received treatment according to PC, while 6 patients (43%) received FPM-guided treatment (Fig. 1, Fig. 3a, Supplemental Materials-Clinical Outcomes). For outcomes reporting, an objective response (OR) was defined as achieving the best overall response of “Partial Response” or “Complete Response” to treatment using RECIST 1.1\textsuperscript{36}.

Remarkably, five of six FPM-guided patients (83%) achieved an OR, and none of the FPM-guided patients had progressive disease as best overall response, having all achieved stable disease or better (Fig. 3a). In contrast, only one of the eight PC-treated patients (13%) achieved an OR, and six of the eight PC patients (75%) continued to have progressive disease (Fig. 3a). Importantly, we observed significant improvement for FPM-guided patients in both the objective response rate (p = 0.0104, Fig. 3a) and progression-free survival (PFS) (p = 0.0020, Fig. 3b) versus patients treated by PC. Additionally, overall PFS was significantly increased in the FPM guided cohort versus each patient’s own previous regimen PFS (p = 0.0001) (Fig. 3c).

Additionally, we assessed the PFS ratio between previous and study regimens to determine the overall impact of FPM-guided and PC treatments, using the PFS ratio $\geq 1.3x$ (a commonly used metric in precision oncology studies) as an indicator of treatment success\textsuperscript{32,33,37}. Significantly more FPM-guided patients than PC-treated patients achieved a PFS ratio $\geq 1.3x$ (p = 0.0107, Fig. 3d, Supplemental Fig. 2e), and the median FPM PFS ratio was 8.5x (range 1.05x – 48x) compared to 1x (range 0.14x – 28x) for PC-treated patients (Fig. 3d). Moreover, two of six FPM-guided patients (EV013 and EV009) experienced exceptional responses, defined as triple the PFS duration compared to expected response duration for the respective disease\textsuperscript{38}.

It is worth noting that, at enrollment, there were no significant differences between treatment cohorts in objective response rate (p = 0.4295, Supplemental Fig. 2a) or in PFS (p = 0.1470, Supplemental Fig. 2b), demonstrating that patients in both cohorts presented with similarly poor outcomes with prior treatments. Furthermore, PC-treated patients did not demonstrate any significant differences in objective response rate (p = 1.0000, Supplemental Fig. 2c) nor in PFS (p = 0.7820, Supplemental Fig. 2d) between study and previous regimens.

Notably, both cohorts received largely standard and readily accessible chemotherapy agents, establishing the utility of the ex vivo functional testing platform to repurpose and prioritize existing approved drugs to overcome drug resistance in heavily pretreated progressive cancers. This data, therefore, indicates that treatment guided by FPM leads to greater outcomes in pediatric cancer patients versus PC.

**DST results correlate with clinical outcomes.**

To determine the predictive ability of our DST platform, we conducted an analysis to assess the clinical correlation between treatment DSS and clinical response. We first examined the association between DSS and type of OR and found a positive relationship between the two (Fig. 3e), as well as a significant difference between treatment DSS from treatment responders and non-responders (p = 0.0012) (Fig. 3e). We also found a significant correlation between treatment DSS and clinical PFS ($\rho = 0.8732, p = 0.0003$,}
Fig. 3f). Finally, we identified the optimal DSS threshold to predict OR using a receiver operating characteristic (ROC) curve (AUC = 1.0000) (Fig. 3g). At the optimal DSS threshold of > 25, there was a strong clinical correlation between DSS and OR, as indicated by high values for all metrics (all metrics = 1.0000) (Fig. 3h).

Apart from the feasibility clinical trial discussed in this study, an additional observational trial focused on newly diagnosed sarcoma patients was undertaken (NCT04956198, Supplemental Materials-Observational Study Data). This study provided supplementary paired data on treatment DSS and clinical outcomes for six additional patients (Supplemental Materials-Correlation Analysis Data). Combining both datasets, we continued to observe a positive relationship between treatment DSS and OR (Supplemental Fig. 4a), again with a significant difference in treatment DSS between treatment responders and non-responders (p = 0.0005) (Supplemental Fig. 4a). We also again identified a significant correlation between treatment DSS and PFS (ρ = 0.7132, p = 0.0006, Supplemental Fig. 4b). Predictive performance of DSS data was confirmed when the six additional patients from the observational study were included (ROC AUC = 0.9464, Precision-Recall AUC = 0.9725) (Supplemental Fig. 4c, 4d). Taken together, these data demonstrate that DSS is a strong predictor of treatment response, with DSS > 25 strongly predicting OR and DSS showing significant positive correlation with PFS. These findings further emphasize the potential of DST as a valuable tool for guiding treatment decisions in high-risk malignancies, including pediatric/adolescent cancers.

Hierarchical Clustering and Analysis of Ex Vivo Functional Response Profiles.

A total of 56 drugs commonly screened across the majority of patients were used to generate hierarchical clusters of ex vivo drug response, identifying two large sub-clusters (Fig. 4a, Supplemental Materials-Dendrogram Input by Drug). However, no particular cancer type was more significantly represented in either cluster, suggesting that patients with similar types of cancer do not exhibit similar responses to individual drugs. This was confirmed through analysis of individual sample DSS profiles (Fig. 4b). We also repeated hierarchical clustering using aggregated DSS scores based on class of drug (Fig. 4c, Supplemental Materials-Dendrogram Input by Mech) and again found no distinct representation of any particular cancer type in either sub-cluster. Interestingly, we did observe an increase in the clustering of leukemias (44% and 20% clustered by overall DSS versus 50% and 10% clustered by DSS aggregated by drug mechanism of action) and sarcomas (33% and 50% clustered by overall DSS versus 30% and 60% clustered by mechanism-aggregated DSS profiles), suggesting that efficacy of a drug’s mechanism of action may be more conserved among cancer types (Fig. 4d). Further analysis revealed four patients with the same cancer subtype were functionally most similar to each other, a significantly greater number than the number of patients clustered by overall DSS (p = 0.0417). Taken together, the data suggest that, while a drug’s mechanism of action may be more associated with efficacy, the response of individual drugs within the same class can still differ among patients. This underscores the importance of personalized treatment plans using functional profiling to guide treatment decisions.
Genomic Tumor Profiling Actionability and Ex Vivo Correlation

Patients had diverse genomic profiles identified through panel sequencing. Of the genomic variants discovered, only six were found in more than three patients including TP53 mutations (six of 20 patients, 30%), CKDN2A/B loss (five of 20 patients, 25%), CBL variants (four of 20 patients, 20%) and a merged Epigenetic Gene Variant signature constructed from epigenetic genes (SMARCA4, SETD2, CREBBP, KMT2D, SMARC1, KMT2C, ASXL2, EZH2, KDM6A, KMT2A-EPS15) (Fig. 5e). CBL variants were of particular interest, as they were not previously reported in pediatric cancer genomics, but have been established in a variant-associated tumor predisposition syndrome (Fig. 5e)\(^39\). In addition, other genetic variants frequently found in cancer were identified including MYC or MYCN mutations (one amplification each, 5%) and disease specific gene fusions including PAX3-FOX01 in alveolar rhabdomyosarcoma (two of two patients, 100%) and EWSR-FLI1 fusions in Ewing’s sarcoma (two of four patients, 50%) (Fig. 5e).

Notably, there were few identified relationships between genomic variants and ex vivo drug sensitivity. Interestingly, disulfiram DSS showed a significant inverse correlation with wild-type CDKN2A/B, suggesting that patients without CDKN2A/B loss may respond more favorably to disulfiram treatment. Additionally, both disulfiram and panobinostat DSS exhibited significant correlations with several Epigenetic Gene Variants (Fig. 5f-g, Supplemental Materials-Complete Genomic Results), suggesting that these specific epigenetic variants may influence response to these drugs. No other significant univariate relationships were identified from panel tumor profiling analysis. These data, however, demonstrate the predictive advantage of combining functional drug testing with genomic profiling.

Only one disease-associated clinically actionable genetic mutation was identified, specifically a FLT3-ITD mutation in a patient (EV013) with acute myeloid leukemia (AML) (Figure S3). For this patient, DST was performed with three FDA-approved FLT3-targeting inhibitors. Testing revealed that midostaurin exhibited the highest efficacy (DSS = 5.97), with sorafenib (DSS = 1.81) and ponatinib (DSS = 0) demonstrating limited effectiveness. Strikingly, DST testing revealed increased cell proliferation with steroid drugs, namely dexamethasone and methylprednisone. Consequently, steroid administration was withheld, and the patient was treated with the FPM-guided combination of midostaurin, cytarabine, and fludarabine. FPM-guided treatment resulted in complete response following just 33 days of treatment, as measured by a minimal residual disease (MRD) of < 0.01%. The patient subsequently underwent an allogeneic bone marrow transplant and is currently in remission, considered an exceptional responder. Notably, the patient's previous regimen only resulted in a similar response after 150 days, demonstrating the capability of an FPM-guided approach to not only provide a more expedient outcome than PC treatment, but in reducing unnecessary risk and drug-related toxicities.

Tumor panel profiling also identified genomic variants associated with demonstrated benefit in different cancer types (4 of 20 patients, 20%) (Fig. 2f, Supplemental Materials-Actionable Panel Seq Results). Additionally, two patients had variants with potential clinical benefits identified prior to enrollment, which did not appear in our own genomic profiling. When assessing all drugs with potential benefit from these
six patients, DST identified no tumor sensitivity (DSS = 0) for any drugs (Supplemental Materials-DSS Panel Result Correlation). Overall, only a drug targeting a directly associated clinically actionable mutation, midostaurin, demonstrated any effectiveness by DST for any patient, revealing a limited relationship between potentially actionable genomic mutations and drug sensitivity.

**Explainable AI/ML provides novel pan-cancer drug response potential biomarkers for pediatric cancer.**

To identify potential multi-omics biomarkers of drug response across the pediatric cancer cohort, we performed whole exome and whole transcriptome sequencing on n = 13 patient samples with available remaining tissue (Supplemental Materials-NGS Samples). We then leveraged an explainable AI/ML (xAI/ML) platform to integrate functional and molecular profiling data around two drugs of interest, idarubicin, and romidepsin (Fig. 5, Supplemental Fig. 5).

Romidepsin selectively inhibits HDAC1 and HDAC2 with weak inhibitory activity against class II HDACs\(^ {40,41} \), and is the only selective HDAC inhibitor currently approved by the FDA for an oncology indication\(^ {42} \). Surprisingly, HDAC1 and HDAC2 exhibited no significant association with DSS values for romidepsin whether individually or paired (Fig. 5a). Among all HDACs, only expression of HDAC3 was significantly associated with romidepsin DSS values (p = 0.0295; correlation \( \rho = 0.7403, p = 0.0087 \)) (Fig. 5b, 5c, Supplemental Fig. 5a, 5b).

The application of the xAI/ML in the analysis of romidepsin revealed two promising hypotheses for multi-omics biomarkers. These hypotheses leverage the expression patterns of HDAC3-COMP (DSS p = 0.0012; correlation \( \rho = 0.8077, p = 0.0014 \)) as well as the mutation status of \( TP53-TXNIP \) (DSS p = 0.0016; correlation \( \rho = 0.8452, p = 0.0016 \)) (Fig. 5b, 5c). The integration of both independent biomarkers further improves the correlation with romidepsin DSS (DSS p = 0.0012; correlation \( \rho = 0.8736, p = 0.0002, \) Fig. 5b, 5c). An additional biomarker was identified which enhanced correlation (\( \rho = 0.9541, \) Supplemental Fig. 5c, 5d, Supplemental Material).

The biomarker HDAC3-COMP has previously been associated with severity and progression in multiple cancers including breast, colorectal, and hepatocellular carcinoma\(^ {43-45} \). Additionally, it has been demonstrated that \( HDAC3 \) can regulate \( COMP \) expression by acetylating histone H3 in the COMP promoter region\(^ {46,47} \). This suggests a potential role for HDAC3-COMP genes as a regulatory factor influencing the efficacy of romidepsin treatment. Interestingly, both \( TP53 \) and \( TXNIP \) are tumor suppressor genes\(^ {48} \) and \( TXNIP \) may act as a regulator of \( TP53 \) in multiple contexts, including breast cancer\(^ {48,49} \). The mutation status of \( TP53-TXNIP \) may play a crucial role in modulating the response to romidepsin treatment, indicating a potential biological mechanism underlying the effectiveness of romidepsin. These two distinct hypotheses shed light on the molecular pathways and genetic factors that may be involved in the response to romidepsin in relapsed/refractory pediatric tumor patients. Furthermore, the genes involved in the two biomarkers form a connected network with HDAC1 and HDAC2 (Fig. 5d), suggesting further biological mechanisms connecting the identified biomarkers of romidepsin response.
Idarubicin is an anthracycline agent which inhibits the activity of the DNA topoisomerase II (TOP2), resulting in extensive DNA fragmentation and consequent antimitotic and cytotoxic effects\textsuperscript{50}. Analysis of TOP2A and TOP2B expression (the reported primary targets for idarubicin\textsuperscript{50}) demonstrated limited correlation with idarubicin sensitivity both individually and combined (Fig. 5e) and were not among the top gene targets identified during idarubicin xAI/ML analysis (Supplemental Fig. 5e).

The xAI/ML analysis of idarubicin identified two promising multi-omics biomarker hypotheses designed around the expression of VEGFA-MECOM-HMGA1 (DSS $p = 0.0012$; correlation $\rho = 0.8082$, $p = 0.0015$), and mutation status in \textit{BCL2-BCL2L13} (DSS $p = 0.0023$; correlation $\rho = 0.8484$, $p = 0.0023$) (Fig. 5f, 5g). Integration of the two independent biomarkers further improves the correlation with idarubicin DSS (DSS $p = 0.0012$; correlation $\rho = 0.9269$, $p < 0.0001$) (Fig. 5f, 5g). All three genes in the VEGFA-MECOM-HMGA1 biomarker are known to be involved in tumorigenesis or tumor progression in multiple cancer types\textsuperscript{51–53}. Interestingly, \textit{VEGFA} polymorphism and expression status has previously been associated with clinical response to idarubicin\textsuperscript{54}, \textit{HMGA1} controls \textit{VEGFA} transcription\textsuperscript{54,55}, \textit{MECOM} controls the \textit{VEGF} signaling pathway\textsuperscript{56}, and \textit{VEGFA} and \textit{HMGA1} are both target genes of the \textit{MECOM} transcription factor\textsuperscript{57}. The expression, mutation status, and interaction of these genes in the context of cancer may influence the efficacy of idarubicin and its impact on tumor growth and progression.

Furthermore, venetoclax (BCL2 inhibitor) has been used in combination with idarubicin for treatment of AML in clinical trials\textsuperscript{58}, and BCL2L13 has been associated with treatment resistance negative outcomes in pediatric ALL\textsuperscript{59}, renders poor prognosis in renal cell carcinoma\textsuperscript{60}, and may be a mitophagy pathway that potentiates triggering of lethal autophagy for idarubicin\textsuperscript{61}. BCL2 and BCL2L13 are both BCL2 family members and share protein domains\textsuperscript{62}. The genes in the independent biomarkers form a connected network around canonical idarubicin targets, suggesting potential interacting biological mechanisms mediating idarubicin response (Fig. 5h).

Overall, the application of xAI/ML analysis enables the discovery of new and actionable multi-omics biomarkers for both novel and repurposed drugs. Understanding the biological significance of these multi-omics biomarkers could potentially aid in patient stratification, treatment selection, and personalized approaches. Additionally, these biomarkers may serve as potential targets for therapeutic interventions and contribute to the development of more effective treatment approaches in childhood cancer. As FPM approaches become increasingly adopted in clinical practice and the availability of paired functional and molecular datasets grows, we anticipate the development of a future collaborative workflow that combines FPM and xAI/ML. This integrated approach will incorporate functional drug response data with molecular profiling and pathway information, serving as the foundation for refining individualized treatments, advancing FPM strategies, and identifying novel predictive biomarkers (Fig. 6).

\textbf{Discussion}
Molecular tumor profiling is rapidly emerging as a customary precision medicine approach for cancer patients who have exhausted standard-of-care. Unfortunately, relying solely on genomic profiling is frequently inadequate for determining an effective treatment strategy, especially due to the incomplete comprehension of the pharmacogenomic mechanisms unique to each patient. Additionally, the relatively few genomic driver mutations in pediatric cancers results in limited utility of genomic profiling in such a high-risk cancer population.

Our clinical study aimed to expand personalized medicine beyond the limits of genome-targeted therapy by providing additional functionally defined individualized therapeutic options to pediatric/adolescent cancer patients who had exhausted previous clinically validated treatments. To achieve this, we integrated genomic profiling with functional DST by testing individual patient-derived tumor samples with a library of FDA-approved drugs. We enrolled 25 patients and were able to generate DST data for 21 patients (84%) and integrate genomic profiling in 19 patients (76%). Among enrolled patients, 14 ultimately received medical intervention, with six of these patients pursuing FPM-guided therapy (43%). Remarkably, five of these six patients (83%) demonstrated improved ORs, with a median 8.5-fold increase in PFS compared to their previous regimen, demonstrating the capability of FPM to improve patient outcomes. Conversely, of eight patients receiving PC salvage treatment, only one patient (13%) achieved an OR, correlating with anticipated outcomes for refractory pediatric/adolescent cancers and emphasizing the need for more refined treatment options.

Recent pioneering studies in Finland and Austria have demonstrated the feasibility of implementing FPM-guided therapies for adult leukemia and lymphoma patients. Our work represents a significant milestone in the advancement of FPM by providing the first prospective study to include both liquid and solid tumors, regardless of cancer type, thereby broadening the scope of usefulness for FPM. Moreover, our study is the first FPM study to address pediatric/adolescent cancer, addressing a critical gap in current treatments. By providing the first prospective FPM study conducted in the United States, we are expanding access to refined personalized treatment options. Our study also demonstrated the integration of FPM and xAI/ML to identify novel biomarkers of many current treatments such as chemotherapies that lack established genomic biomarkers for patient selection.

Large-scale genomics precision medicine trials for adult cancers over the past decade reported objectives responses in ~3% of enrolled patients and ~8.6% of evaluable patients (Supplemental Fig. 6), similar to recent updates from the NCI-MATCH which reported objective responses in 1.1% of enrolled patients and 10% of evaluable patients. In comparison, the limited interventional FPM studies (including our current work) have now reported 20% of enrolled patients and 57% of evaluable patients achieved an objective response when guided by FPM data. While limited in total patient accrual, the initial clinical signal from FPM studies is striking compared to 10+ years outcomes from genomics precision medicine studies.

We also acknowledge the limitations inherent in our study. One notable limitation to including a wide variety of solid and liquid cancer types is a failure to collect extensive outcome data for any particular
cancer type, limiting our ability to statistically compare outcomes. To evaluate the overall effect across heterogeneous diseases and treatment regimens, we adopted a PFS ratio as the primary endpoint, an approach common in precision medicine trials where each patient serves as their own control\textsuperscript{32,33,74,75}. Another limitation is inherent to our inclusion of relapsed and refractory patients, namely that exposure to extensive treatments prior to enrollment may have limited patients’ abilities to respond. Despite the lack of a randomized control group and our relatively small cohort, our results suggest a broad range of effective chemotherapeutic drugs and targeted inhibitors are capable of overcoming drug resistance, even in heavily refractory cancers, provided that the appropriate drugs are selected for each patient. Additional barriers remain to the successful application of FPM as a whole, including the consideration of off-label use for potentially effective therapeutic options and the financial limitations that can impact treatment choice. Overcoming these obstacles will require collaboration between regulatory bodies, non-profit and for-profit organizations to advance the field of personalized medicine.

Overall, our approach to personalized medicine demonstrates immense promise to expand treatment options for patients with limited viable alternatives. Our ability to screen multiple monotherapy and combination options with high clinical accuracy, and to provide drug response data within a clinically actionable timeframe, has the potential to improve the lives of cancer patients previously failed by standard clinical care. The findings in this study provide evidence supporting the feasibility and efficacy of FPM approaches, indicating the need for continued validation to make these approaches accessible to a wide range of cancer patients with unmet clinical needs. The observed increase in objective response rate, significant improvements in overall PFS, and the proportion of patients with a high PFS ratio highlights the importance of continued clinical development of our FPM approach, with the ultimate goal involving randomized, multi-site, controlled clinical trials contrasting PC therapy, genomics-guided therapy, and integrated FPM-guided therapy.

The turnaround time and cost for genomic tumor profiling has now reached an inflection point where routine clinical use is possible for an increasing number of cancer patients. With the recent reports from Europe on FPM for hematological cancer and our results on FPM in both hematological and solid pediatric/adolescent cancer, \emph{ex vivo} tumor drug sensitivity testing is also reaching the critical inflection point where turnaround time and cost now enable widespread clinical use, and can be used to support even high-risk patients with immediate clinical need. The potent capacity for FPM approaches to expand treatment choices for patients with limited options is evident. The promising results obtained thus far warrant further validation and support the need for ongoing development to establish the role of FPM in routine clinical care to improve patient outcomes.

Methods

Patient Samples and Tumor Processing

Samples were collected from 24 of 25 consented pediatric/adolescent patients with refractory solid or hematological cancers following informed consent obtained from all legal guardians of patients, in
accordance with the Western Institutional Review Board (IRB), IRB number 20181421. All primary tumor samples were collected fresh and sent to our lab for processing within 24–48 hours.

Samples from solid cancers were dissociated from primary tissue as previously described\(^{30,76}\) and resuspended in RPMI 1640 medium (Gibco) supplemented with antibiotics (100U ml\(^{-1}\) penicillin and 100µg ml\(^{-1}\) streptomycin) and 10% FBS. Mononuclear cells were isolated from hematological cancers using SepMate PBMC Isolation Tubes (StemCell) according to the manufacturer's instructions as previously described\(^{31,77}\) and resuspended in Mononuclear Cell Medium with Supplement (PromoCell). All patient cells were cultured for a minimum of 12hrs before proceeding with drug sensitivity testing.

**Drug Sensitivity Testing**

A custom drug library encompassing formulary drugs from Nicklaus Children's Hospital (Miami, FL), as well as non-formulary FDA-approved cancer drugs and phase III or IV oncology drugs was purchased (ApexBio). Drugs were tested at a range of 10 concentrations from 10µM to 0.5nM in duplicate\(^{30}\). Additional drugs were added to the drug library for specific patients at the request of the physician. A few patients underwent partial library testing when the size of tumor sample was small. Cells from solid tumor samples were harvested using TrypLE Express (Gibco). Mononuclear cells from hematological cancers or dissociated cells from solid tumors were dispensed in white 384-well microplates (ThermoFisher) at 500–2000 cells/well. Drugs at the designated concentrations were added to cells using an epMotion P5073 liquid handler (Eppendorf). The chemical compounds DMSO (negative control) and benzethonium chloride (positive control) were also added. All cells were subsequently incubated at \(37°C\) and 5% CO2 for 72hrs. Cell viability was assessed following 72hrs by evaluating ATP levels in cells using CellTiter-Glo or CellTiter-Glo 3D luminescent cell viability assay (Promega) according to the manufacturer’s protocol. Luminescence was measured using a multimode plate reader (Perkin Elmer). The resulting luminescence data were used to generate dose-response curves to derive Drug Sensitivity Scores (DSS) using GraphPad Prism 9.0 and the DSS package in R version 3.6.3, as previously described\(^{30,77,78}\).

**Genomic Panel Sequencing**

Formalin-fixed paraffin-embedded (FFPE)-preserved tissue sections obtained following surgical resection and matched whole blood from patients were sent to the UCSF Clinical Cancer Genomics Lab for UCSF500 Cancer Gene Panel sequencing. All patients enrolled in the study underwent genomic tumor profiling if sufficient tissue was available. In addition, several patients underwent previously accessed genomic panel sequencing services through Foundation Medicine or CHLA OncoKids; we report results from sequencing services performed outside the clinical study, when available. Analyte isolation, physical sequencing, and clinical interpretation were performed by each respective service.
Pediatric/Adolescent Functional Precision Medicine
Molecular Tumor Board (FPMTB)

Results from Drug Sensitivity Testing and genomic panel sequencing for each patient were made available as soon as possible to the FPM Tumor Board (FPMTB) consisting of physicians managing the patients, pharmacists, hematology/oncology nurses, clinical research coordinators from Nicklaus Children’s Hospital as well as translational researchers familiar with the functional assays from Florida International University. Upon receiving the results, the FPMTB convened to evaluate the data, consider the availability of candidate drugs for off-label use, and review the treatment histories of each patient. Subsequently, a final list of therapeutic options, ranked in order of preference along with recommended doses and schedules, was provided for each patient\textsuperscript{30}. The board also did follow-up analysis of treatment responses for eligible patients. More details for patient, treatment selection and outcomes are shown in Supplemental Materials-Clinical Outcomes.

Hierarchical Clustering and Spearman correlation coefficient analysis of Drug Sensitivity Data

The hierarchical clustering analysis of ex vivo patient DST profiles was performed using the R statistical software version 3.6.3\textsuperscript{40} using the dist, tree, and pheatmap packages. In brief, ex vivo DSS data were logarithmically transformed using a log2 scale. The distances between patient profiles were calculated using the Euclidean distance measure, and clusters were agglomerated using complete linkage. The clustering analysis was performed on $n = 20$ patient sample profiles using DSS results from $n = 56$ distinct agents successfully tested on all 20 patient samples. One patient sample (EV021) was excluded from the analysis due to having a smaller library of tested agents.

Individual drug DSS values for each patient were then averaged into a set of drug classes, which were defined based on established drug mechanisms: alkylating agents, antimetabolites, antimitotics, antitumor antibiotics, HDAC inhibitors, immunomodulators, kinase inhibitors, miscellaneous antineoplastic, proteasome inhibitors, rapalogs, Topoisomerase I or II inhibitors, and drugs with other mechanisms. The resulting average DSS values for each drug class were log2 transformed, and hierarchical clustering was performed again using these transformed values, following the same methodology described earlier.

One-to-one similarity between patient sample DSS profiles at the individual drug level and the aggregated mechanism level was quantified via Spearman correlation coefficient analysis using the “cor” function in the corrplot library, which was also used to plot one-to-one correlation coefficients. Cancer indication for each patient was compared with the indication for the sample with the highest Spearman correlation coefficient to determine if the two indications matched amongst samples with the highest correlation.

Whole-exome and whole-transcriptome sequencing and analysis
DNA and RNA isolation for sequencing of hematological cancer samples was performed using Qiagen DNA and RNA Mini-Prep kits per manufacturer’s instructions (Qiagen). Frozen isolated DNA and RNA were shipped overnight for physical sequencing at Beijing Genomics Institute (BGI).

DNA and RNA isolation for solid tumor samples was performed from sectioned FFPE tissue stored at Nicklaus Children’s Hospital. Tissue sectioning performed by HisoWiz (New York, NY). BGI performed analyte isolation from FFPE curls. FFPE tissues were shipped to BGI at ambient temperatures in an independent package.

Sequencing data provided by BGI were analyzed using previously established analysis pipelines. In brief, raw FASTQ sequencing files from DNA sequencing were quality control filtered via SOAPnuke and aligned to the GRCh38 human reference genome using BWA MEM aligner. Somatic mutations and indels were called using Genome Analysis Toolkit (GATK) Version 4.0 according to best practices for tumor-only samples, and copy number alterations were called using VarScan2, with sex-matched non-malignant samples (NA12878 [Female] and NA12865 [Male]) to serve as copy number baseline samples. Post-QC RNA sequencing data were aligned to the reference transcriptome using the STAR aligner, and gene expression was quantified using RSEM while gene fusion events were detected using STAR-Fusion.

**Explainable AI/ML Biomarker Analysis**

The explainable AI/ML (xAI/ML) platform to identify causal pharmacogenomic biomarkers of drug response is adapted from the PTIM-Circuit personalized combination platform and the PTM-Biomarker analysis platform. In brief, patient-derived DSS profiles and whole exome and whole transcriptome sequencing data were ingested into the xAI/ML framework to generate multi-omics relationships differentiating ex vivo drug sensitivity. Data from whole exome sequencing consisted of called mutations, indels, and copy number changes. Data from whole transcriptome sequencing consisted of gene expression (normalized via the GeTMM processing) and gene fusion events. Top gene features were aggregated to create networks of differential gene features linked by common biological properties as defined by interaction network and gene ontology analyses. Gene network analysis was performed in Cytoscape 3.9.1 using the GeneMania package.

**Statistics**

Kaplan-Meier curve generation and analysis were performed in GraphPad Prism 9.0. Barnard’s unconditional test of superiority was performed using the Barnard v1.8 package in R version 3.6.3. The exact binomial test was performed in R version 3.6.3. Cox regression with clustered computation was performed in R version 3.6.3 using the “coxph” function in the “survival” package. Mann-Whitney U-tests, Kolmogorov-Smirnov tests, McNemar’s test with continuity correction, Kruskal-Wallis test, simple linear regression, Spearman correlation coefficient analysis for DSS-PFS correlation/DSS-biomarker correlation, and the Wilcoxon matched pairs tests were performed in GraphPad Prism 9.0. One-to-one DSS profile Spearman correlation coefficient analysis was performed in R version 3.6.3 using the “corrplot” package.
Statistical tests, uses, results, and software packages are further described in Supplemental Materials-
Statistical Tests and Tools.

**Abbreviations**

FPM: Functional Precision Medicine

FPMTB: FPM Tumor Board

PC: Physician's choice

AI/ML: Artificial Intelligence/Machine Learning

xAI/ML: Explainable Artificial Intelligence/Machine Learning

OR: Objective Response

ORR: Objective response rate

PFS: Progression-Free Survival

PFS2/PFS1: ratio of PFS between regimen on trial (PFS2) and regimen before trial (PFS1)

DST: Drug Sensitivity Testing

DSS: Drug Sensitivity Scores

CR: Complete Response

PR: Partial Response

SD: Stable Disease

PD: Progressive Disease

CNS: Central Nervous System

ALL: Acute Lymphoblastic Leukemia

AML: Acute Myeloid Leukemia

AST: Astrocytoma

EP: Ependymoma

EWS: Ewing’s Sarcoma
GBM: Glioblastoma Multiforme
MRT: Malignant Rhabdoid Tumor
MB: Meduloblastoma
NB: Neuroblastoma
OS: Osteosarcoma
RMS: Rhabdomyosarcoma
WT: Wilms’ tumor
AUC: Area under curve
MCC: Matthew’s Correlation Coefficient
FFPE: Formalin fixed, paraffin embedded

Declarations

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**Availability of Data and Materials**

All materials used in our analysis are provided as tables in supplemental materials. Raw sequencing data is available through dbGaP (data submission and accession ID generation is currently underway).

**Conflict of Interest**

NEB is co-founder of and holds shares in First Ascent Biomedical.

The remaining authors declare no conflicts of interest.

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**References**


92. Smid, M., et al. Gene length corrected trimmed mean of M-values (GeTMM) processing of RNA-seq data performs similarly in intersample analyses while improving intrasample comparisons. *BMC*


**Figures**

**Figure 1**

**CONSORT flow diagram depicting the FPM workflow for enrolled patients.** FPM workflow including patient enrollment, biopsy/resection, functional ex vivo drug sensitivity testing and molecular tumor profiling, and report delivery to the FPMTB for clinical decision-making. Numbers at each exit and endpoint represent patient counts.
Figure 2

**FPM workflow is feasible and actionable in a clinically relevant timeframe.** a) Histogram of patient ages at enrollment. b) Distribution of biological sex of enrolled patients. c) Distribution of ethnicity of enrolled patients. d) Patient enrollment by diagnosed disease type: ALL (Acute Lymphoblastic Leukemia), AML (Acute Myeloid Leukemia), AST (Astrocytoma), EP (Ependymoma), EWS (Ewing’s Sarcoma), GBM (Glioblastoma Multiforme), MRT (Malignant Rhabdoid Tumor), MB (Medulloblastoma), NB
(Neuroblastoma), OS (Osteosarcoma), RMS (Rhabdomyosarcoma), WT (Wilms' tumor). e) Outcomes of patient sample testing through DST and genomics approaches distributed by cancer type (Hematological [Heme], Sarcoma [Sarc], CNS, and Renal). f) Distribution of patients with therapeutic options identified through DST profiling and genomic profiling by cancer type (Hematological [Hem], Sarcoma [Sarc], CNS, and Renal). Genomics = genetic lesion matched to drug approved in patient's disease type, Genomics (Potential) = genomic lesion matched to drug approved in other disease types. g) Distribution of turnaround time in days for hematological cancer DST assays, solid cancer DST assays, and UCSF500 genomics assays.

Figure 3

FPM provides significant clinical benefit in refractory/relapsed pediatric cancer patients. a) Swimmer plot showing clinical and molecular characteristics of patients as well as patient responses to treatments assigned following FPMTB review, grouped by FPM-guided and PC-treated cohorts. * denotes the patient
that received hematopoietic stem cell transplantation following FPM-guided therapy. ALL = Acute Lymphoblastic Leukemia, AML = Acute Myeloid Leukemia, EWS = Ewing’s Sarcoma, GBM = Glioblastoma Multiforme, MB = Medulloblastoma, NB = Neuroblastoma, OS = Osteosarcoma, RMS = Rhabdomyosarcoma. b) Kaplan-Meier survival curves of therapeutic regimens in the PC-treated cohort versus the FPM-guided cohort. c) Kaplan-Meier survival curves of therapeutic regimens in the FPM-guided cohort versus their own previous regimen. d) Before and after plot of the PFS of previous regimen and trial regimen, grouped by PC-treated cohort (left) and FPM-guided cohort (right). Intra-group p-values are Wilcoxon paired samples test, while intergroup p-values are a comparison of the number of patients with PFS ratio ≥ 1.3x (top) and a PFS ratio ≥ 1.3x, while patients with red dots did not. * indicates five PC patients had the same previous and study regimen PFS. Black dots represent the PFS of previous regimen for both cohorts. e) Distribution of DSS scores separated by response type (left) and response class (NR = Non-Responder, R = Responder) in patients reviewed by the FPMTB patients. f) Plot of the relationship between PFS and DSS scores of associated treatments (CR is complete response, PR is partial response, SD is stable disease, and PD is progressive disease) in FPM-guided patients. Blue dashed line represents a line of simple linear regression g) ROC curve of true positive rate and false positive rate of DSS-based response prediction. h) Confusion matrix and associated statistical values of DSS predicted and actual objective response in FPM-guided patients and PC patients at optimal threshold (DSS > 25). Prediction performance metrics (MCC, F1, Accuracy, Precision, Recall) are provided below the confusion matrix.
Figure 4

Post-hoc analysis of patient DSS and genomic tumor panel profiles. a) Agglomerative hierarchical clustering of ex vivo drug sensitivity profiles of 20 DST-assayed patients across 56 common drugs. b) Inter-patient Spearman correlation coefficients of DSS response profiles. Correlation coefficients are visualized as squared values for visual clarity. c) Agglomerative hierarchical clustering of ex vivo drug sensitivity profiles of 20 DST-assayed patients across 56 common drugs, grouped by drug class. d) Inter-patient Spearman correlation coefficients of DSS response profiles grouped by drug class. Correlation coefficients are visualized as squared values for visual clarity. * represents patient DSS profiles most correlated with a patient sample of the same indication. e) Genomic landscape of variants identified through genomic tumor panel profiling via UCSF500. Genes with alterations in two or more patient samples are reported. f) Heatmap of multiple comparison corrected by p-values of univariate
relationships between variant features and drug response. Analysis was limited to gene features present in three or more patient samples. g) Distribution of DSS values for statistically significant relationships between drug sensitivity and tumor panel sequencing features: disulfiram DSS – *CDKN2A/B* status, disulfiram DSS – Epigenetic Variant status, panobinostat DSS – Epigenetic Variant status.

**Figure 5**

- **a)** HDAC1 Exp and HDAC2 Exp
- **b)** Romidepsin Multi-Omics Biomarkers
- **c)** HDAC3 Exp and HDAC3 + COMP
- **d)** Romidepsin Biomarker Network
- **e)** TOP2A Exp and TOP2B Exp
- **f)** Idarubicin Multi-Omics Biomarkers
- **g)** VEGF-VEGFA-HMGA1 and BCL2-BCL2L13
- **h)** Idarubicin Biomarker Network
Post-hoc explainable AI/ML analysis of DSS and multi-omics data. a) DSS distribution by low or high gene expression level of canonical romidepsin targets (HDAC1 and HDAC2) as well as HDAC3. Pearson correlation coefficient and p-value is provided alongside distribution plots. High and low expression threshold is based on median cohort gene expression. b) Prioritized multi-omics biomarker hypotheses of romidepsin response, including individual and merged biomarker scores. Heatmap of gene expression is visualized around median expression. c) DSS distribution of individual and merged multi-omics romidepsin response biomarkers identified by xAI/ML analysis. Pearson correlation coefficient and p-value is provided alongside distribution plots. d) Interaction and association network for canonical romidepsin targets and genes identified in romidepsin response biomarkers. e) DSS distribution of reported idarubicin targets (TOP2A and TOP2B) by low or high gene expression level. Pearson correlation coefficient and p-value is provided alongside distribution plots. High and low expression threshold is based on median cohort gene expression. f) Prioritized multi-omics biomarker hypotheses of idarubicin response, including individual and merged biomarker scores. Heatmap of gene expression is visualized around median expression. g) DSS distribution of individual and merged multi-omics idarubicin response biomarkers identified by xAI/ML analysis. Pearson correlation coefficient and p-value is provided alongside distribution plots. h) Interaction and association network for canonical romidepsin targets and genes identified in romidepsin response biomarkers. P-values above all distribution plots are from Mann-Whitney U tests.

Figure 6

Integration of FPM and xAI/ML for advancing personalized medicine workflows. Workflow diagram depicting the sequential process of the FPM and xAI/ML approach for enhancing individualized cancer medicine. Patients are enrolled followed by a biopsy/resection of the tumor sample. Live patient-derived cells undergo high-throughput ex vivo DST assay in combination with molecular tumor profiling using
whole-exome sequencing and whole-transcriptome sequencing. The results of both the DST and molecular profiling are reported to the FPM tumor board to make informed treatment decisions based on each individual patient’s profile. In parallel, the xAI/ML platform analyzes the DST results, the molecular profiling data, and existing knowledge on drug interactions to generate potential drug combinations tailored to each patient's specific tumor characteristics and to uncover potential multi-omics biomarkers. The drug combination rankings will also be reported to the FPM tumor board for treatment decision-making. The process will enable the FPM tumor board to make treatment decisions in a clinically actionable timeframe (less than 2 weeks) for each individual patient. The workflow shows the multidimensional and personalized approach for further development of personalized cancer medicine.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FPMSU1.xlsx
- FPMSupplementaryFigures07022023.docx